Attorney Docket No.: A33002-PCT-USA-072667.0124

**PATENT** 

## WHAT IS CLAIMED:

- 1. Nucleic acid fragment, characterized in that it comprises a nucleic acid sequence coding for an androctonine.
- 2. Nucleic acid fragment according to claim 1, characterized in that it is a sequence of DNA.
- 3. (Amended) Nucleic acid fragment according to claim 1, characterized in that the androctonine consists of a peptide which can be produced by and isolated from scorpions, in particular the species *Androctonus australis*, the said peptide comprising at least 20 amino acids, preferably at least 25 amino acids, and 4 cysteine residues which form disulphide bridges between themselves.
- 4. (Amended) Nucleic acid fragment according to claim 1, characterized in that the androctonine essentially comprises the peptide\_sequence of general formula (I) below:

**(I)** 

in which Xaa represents a peptide residue comprising at least 1 amino acid,

Xab represents a peptide residue comprising at least 5 amino acids,

Xac represents a peptide residue comprising at least 5 amino acids,

Xad represents a peptide residue comprising at least 3 amino acids, and

Xae represents a peptide residue comprising at least 1 amino acid.

5. Nucleic acid fragment according to claim 4, characterized in that Xab and/or Xad and/or Xae comprise at least one basic amino acid.

- 6. Nucleic acid fragment according to claim 5, characterized in that the basic amino acids are chosen from lysine, asparagine and homoasparagine.
- 7. (Amended) Nucleic acid fragment according to claim 4, characterized in that Xaa represents the peptide sequence Xaa'-Val, in which Xaa' represents NH<sub>2</sub> or a peptide residue comprising at least 1 amino acid, and/or

Xab represents the peptide sequence -Arg-Xab'-Ile, in which Xab' represents a peptide residue of 3 amino acid, and/or

Xac represents the peptide sequence –Arg-Xac'-Gly, in which Xac' represents a peptide residue of 3 amino acid, and/or

Xad represents the peptide sequence –Tyr-Xad'-Lys, in which Xad' represents a peptide residue of 1 amino acid, and/or

Xae represents the peptide sequence –Thr-Xae', in which Xae' represents COOH or a peptide residue comprising at least 1 amino acid.

- 8. Nucleic acid fragment according to claim 7, characterized in that Xaa' represents the peptide sequence –Arg-Ser-, and/or Xab' represents the peptide sequence –Gln-Ile-Lys-, and/or Xac' represents the peptide sequence –Arg-Arg-Gly-, and/or Xad' represents the peptide residue –Tyr-, and/or Xae' represents the peptide sequence –Asn-Arg-Pro-Tyr.
- 9. (Amended) Nucleic acid fragment according to one of claims 1 to 8, characterized in that the androctonine is represented by the peptide sequence of 25 amino acids described by the sequence identifier No. 1 (SEQ ID NO. 1) and the homologous peptide sequences.
- 10. Nucleic acid fragment according to claim 9, characterized in that it is represented by the sequence identifier No. 1 (SEQ ID NO. 1), a homologous sequence or a sequence complementary to the said sequence.

Attorney Docket No.: A33002-PCT-USA-072667.0124

PATENT

19. (Amended) Chimeric gene comprising a coding sequence and heterologous regulation elements in positions 5' and 3' which can function in a host organism, in particular plant cells or plants, these elements being functionally linked to the said coding sequence, characterized in that the said coding sequence comprises at least one DNA fragment coding for androctonine as defined according to claim 1.

- 20. Chimeric gene according to claim 19, characterized in that the host organism is chosen from bacteria, for example *E. coli*, yeasts, in particular yeasts of the genera *Saccharomyces* or *Kluyveromyces*, *Pichia*, fungi, in particular *Aspergillus*, a baculovirus, and plant cells and plants.
- 21. (Amended) Chimeric gene according to claim 19, characterized in that it is combined with a selection marker adapted to the transformed host organism.
- (Amended) Cloning or expression vector for the transformation of a host organism,characterized in that it comprises at least one chimeric gene as defined according to claim19.
- 23. (Amended) Process for transforming host organisms by incorporating at least one nucleic acid fragment or one chimeric gene as defined in claim 19.
- 24. (Amended) Process according to claim 23, characterized in that the chimeric gene is incorporated by means of a vector.
- 25. (Amended) Process according to claim 23, characterized in that the host organism is chosen from bacteria, for example *E. coli*, yeasts, in particular yeasts of the genera *Saccharomyce* or *Kluyveromyces*, *Pichia*, fungi, in particular *Aspergillus*, a baculovirus, and plant cells and plants
- 26. Process according to claim 25, characterized in that the host organism is a plant cell.

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- 27. Process according to claim 26, characterized in that plants are regenerated from the plant cell.
- 28. (Amended) Transformed host organism, in particular plant cell or plant, characterized in that it comprises a chimeric gene defined according to claim 19.
- 29. Host organism according to claim 28, characterized in that it is chosen from bacteria, for example *E. coli*, yeasts, in particular yeasts of the genera *Saccharomyce* or *Kluyveromyces*, *Pichia*, fungi, in particular *Aspergillus*, a baculovirus, and plant cells and plants.
- 30. Plants, characterized in that they comprise transformed plant cells according to claim 29.
- 31. Plant according to claim 30, characterized in that it is regenerated from the transformed plant cell.
- 32. Plant, characterized in that it is obtained from the cultivating and/or crossing of the regenerated plants according to claim 31.
- 33. (Amended) Plant according to claim 30, characterized in that it is chosen from corn, wheat, rapeseed, soybean, rice, sugar cane, beetroot, tobacco, and cotton.
- 34. (Amended) Plant according to claim 30, characterized in that it is resistant to fungal diseases such as those caused by *Cercospora*, in particular *Cercospora beticola*, *Cladisporium*, in particular *Cladisporium herbarum*, *Fusarium*, in particular *Fusarium culmorum* or *Fusarium graminearum*, or by *Phytophthora*, in particular *Phyophthora cinnamoni*.
- 35. (Amended) Plant seeds according to claim 30.
- 39. (Amended) Process for preparing the androctonine, comprising the steps of cultivating the transformed host organism defined according to claim 28 in an appropriate cultivation environment, followed by the extraction and total or partial purification of the androctonine obtained.

Abstract of the Disclosure

The invention concerns a DNA sequence coding for androctonine, a vector containing same for transforming a host organism and the transformation method. More particularly the invention concerns the transformation of plant cells and plants, the droxomycine produced by the transformed plants providing them with resistance to diseases, in particular those of fungal origin.--

Please <u>replace</u> the paper copy of the Sequence Listing previously filed with the paper copy of the Sequence Listing enclosed herewith, inserting the Sequence Listing into the specification after the Abstract of the Disclosure.

## IN THE DRAWINGS:

Please <u>replace</u> the five figures contained on the two sheets of drawings with the substitute figures contained on the five sheets of drawings enclosed herein.

## IN THE CLAIMS:

Please cancel Claims 1-3.

Please amend Claims 4-10, 19-35 and 39 as follows:

4. (Twice Amended) An isolated nucleic acid fragment encoding a polypeptide comprising the polypeptide sequence of general formula (I) below:

Xaa-Cys-Xab-Cys-Xac-Cys-Xad-Cys-Xae (SEQ ID NO.:15)

**(I)** 

wherein Xaa represents a peptide residue comprising at least one amino acid,

Xab represents a peptide residue comprising at least five amino acids,

Xac represents a peptide residue comprising at least five amino acids,

Xad represents a peptide residue comprising at least three amino acids, and

Xae represents a peptide residue comprising at least one amino acid.

5. (Amended) The nucleic acid fragment of Claim 4, wherein Xab and/or Xad and/or Xae comprise at least one basic amino acid.

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- 6. (Amended) The nucleic acid fragment according to Claim 5, wherein the basic amino acids are selected from the group consisting of lysine, asparagine and homoasparagine.
- 7. (Twice Amended) The nucleic acid fragment of Claim 4, wherein: Xaa represents the peptide sequence Xaa'-Val, wherein Xaa' represents NH<sub>2</sub> or a peptide residue comprising at least one amino acid, and/or

Xab represents the peptide sequence –Arg-Xab'-Ile, wherein Xab' represents a peptide residue of three amino acid, and/or

Xac represents the peptide sequence -Arg-Xac'-Gly, wherein Xac' represents a peptide residue of three amino acid, and/or

Xad represents the peptide sequence -Tyr-Xad'-Lys, wherein Xad' represents a peptide residue of one amino acid, and/or

Xae represents the peptide sequence –Thr-Xae', wherein Xae' represents COOH or a peptide residue comprising at least one amino acid.

- 8. (Amended) The nucleic acid fragment of Claim 7, wherein Xaa' represents the peptide sequence –Arg-Ser-, and/or Xab' represents the peptide sequence –Gln-Ile-Lys-, and/or Xac' represents the peptide sequence –Arg-Arg-Gly-, and/or Xad' represents the peptide residue –Tyr-, and/or Xae' represents the peptide sequence –Asn-Arg-Pro-Tyr.
- 9. (Twice Amended) The isolated nucleic acid fragment comprising the nucleic acids encoding the peptide sequence of SEQ ID NO.:2 or homologous peptide sequences.

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10. (Amended) The nucleic acid fragment of Claim 9, comprising the nucleic acid sequence of SEQ ID NO.:1, a nucleic acid sequence homologous to SEQ ID NO.:1, or a nucleic acid sequence complementary to SEQ ID NO.:1.

19. (Twice Amended) A chimeric gene comprising a nucleic acid sequence according to any one of Claims 4 to 10 operably linked to heterologous regulatory elements that are functional in a host organism.

- 20. (Amended) The chimeric gene of Claim 19, wherein the host organism is selected from the group consisting of a bacterium, an E. coli bacterium, a yeast, a yeast of the genera Saccharomyces, a yeast of the genera Kluyveromyces, a yeast of the genera Pichia, a fungus, an Aspergillus fungus, a plant cell, and a plant.
- 21. (Twice Amended) The chimeric gene of Claim 19 further comprising a gene encoding a selectable marker adapted for the transformation of said host organism.
- 22. (Twice Amended) A vector comprising the chimeric gene of Claim 19.
- 23. (Twice Amended) A method for transforming a host organism comprising incorporating the chimeric gene of Claim 19 into the genome of said host organism.
- 24. (Twice Amended) The method of Claim 23, wherein the chimeric gene is incorporated into the genome of the host organism by means of a vector.

- 25. (Twice Amended) The method Claim 23, wherein the host organism is selected from the group consisting of a bacterium, an E. coli bacterium, a yeast, a yeast of the genera Saccharomyces, a yeast of the genera Kluyveromyces, a yeast of the genera Pichia, a fungus, an Aspergillus fungus, a plant cell, and a plant.
- 26. (Amended) The method of Claim 25, wherein the host organism is a plant cell.
- 27. (Amended) The method of Claim 26, wherein a plant is regenerated from the plant cell.
- 28. (Twice Amended) A host organism comprising the chimeric gene of Claim 19.
- 29. (Amended) The host organism of Claim 28, wherein the host organism is selected from the group consisting of a bacterium, an *E.* coli bacterium, a yeast, a yeast of the genera *Saccharomyces*, a yeast of the genera *Kluyveromyces*, a yeast of the genera *Pichia*, a fungus, an *Aspergillus* fungus, a plant cell, and a plant.
- 30. (Amended) The host organism of Claim 29, wherein the host organism is a plant.
- 31. (Amended) The host organism of Claim 30, wherein said host organism is regenerated from a plant cell comprising the chimeric gene of Claim 19.
- 32. (Amended) A plant comprising the chimeric gene of Claim 19 obtained from the cultivating and/or crossing of the host organism of Claim 31.

- 33. (Twice Amended) The plant of Claim 32, wherein the plant is selected from the group consisting of a corn plant, a wheat plant, a rapeseed plant, a soybean plant, a rice plant, a sugar cane plant, a beetroot plant, a tobacco plant and a cotton plant.
- 34. (Twice Amended) The plant of Claim 33, wherein the plant is resistant to fungal diseases selected from the group consisting of those diseases caused by *Cercospora*, *Cercospora* beticola, Cladisporium, Cladisporium herbarum, Fusarium, Fusarium culmorum, Fusarium graminearum, Phytophthora, and Phyophthora cinnamoni.
- 35. (Twice Amended) Seeds from the plants of Claim 32, wherein the seeds comprise the chimeric gene of Claim 19.
- 39. (Twice Amended) A method for preparing the product of the chimeric gene of Claim 19 comprising the steps of cultivating the host organism of Claim 28 in an appropriate cultivation environment, extracting the product of said chimeric gene, and partially or totally purifying the product of said chimeric gene.

## Please add the following new claims:

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- 40. (New) Seeds from the plants of Claim 33, wherein the seeds comprise the chimeric gene of Claim 19.
- 41. (New) The vector of Claim 22, wherein the vector is selected from the group consisting of a plasmid and a recombinant baculovirus.
- 42. (New) The vector of Claim 41, wherein the virus is a baculovirus.